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Novel Camptothecin Derivatives. Part 1: Oxyalkanoic Acid Esters of Camptothecin and Their In Vitro and In Vivo Antitumor Activity

Li-Xi Yang,[a,b,*] Xiandao Pan[a] and Hui-Juan Wang[a]

[a]*Radiobiology Laboratory, California Pacific Medical Center Research Institute, San Francisco, CA 94118, USA*
[b]*St. Mary's Medical Center, San Francisco, CA 94117, USA*

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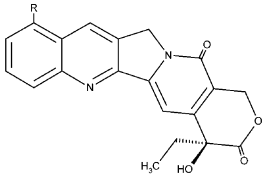
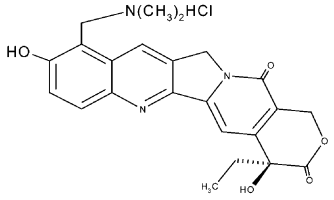
Abstract—A series of oxyalkanoic acid esters of (20*S*)-camptothecin derivatives was prepared by the method of acylation. Their antitumor activity was evaluated on cancer cells in vitro by the colony formation assay and in vivo. These newly synthesized derivatives show a dramatically higher chemotherapeutic activity in killing human cancer cells than the parent drug, camptothecin, and clinically available drugs, irinotecan and taxol. © 2002 Elsevier Science Ltd. All rights reserved.

Camptothecin (**1**) is an alkaloid first isolated from the wood and bark of *Camptotheca acuminata* (Nyssaceae) by Wall and co-workers in 1966.[1] It has been well demonstrated that camptothecin has antitumor activity against many human solid tumors. Mechanistic studies have shown that its antitumor activity is closely associated with its inhibition of topoismerase I, which is required for DNA replication and RNA transcription in cells.[2] However, severe toxicity (e.g., neutropenia, thromobocytopenia, hemorrhagic cystitis, and G.I. symptoms with significant diarrhoea) of camptothecin greatly limited its use in cancer therapy. Many attempts have been made to modify camptothecin's structure in order to reduce its toxicity and maintain or increase its anticancer activity.

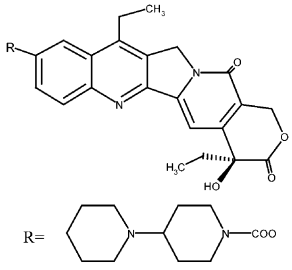
During past decades two groups of camptothecin derivatives have been developed and entered into clinical trials. The first group includes water-insoluble camptothecins, including 9-nitrocamptothecin (**2**)[3] and 9-aminocamptothecin (**3**).[4] The second group, water-soluble, consists of topotecan (**4**)[5] and irinotecan (**5**).[6]



R = H **1**
R = NO_2 **2**
R = NH_2 **3**



Topotecan **4**



Irinotecan **5**

*Corresponding author. Fax: +1-415-750-6215; e-mail: yang@cooper.cpmc.org

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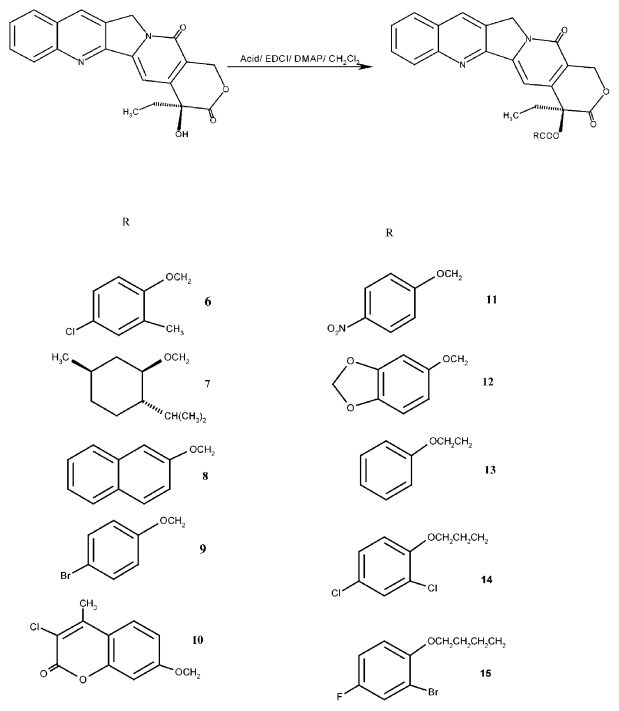
Structure–activity relationship studies suggested that the intact lactone ring E of camptothecin is the most critical structural feature with respect to antitumor activity.[7,13] The severe toxicity of camptothecin may be largely due to the rapid opening of the intact lactone ring when the drug enters the plasma. Studies by Cao et al.[8] showed that the biological life span of lactone forms of their simple camptothecin esters in human and mouse plasma significantly increased when compared with their mother compounds.



Scheme 1.

We hypothesized that the opening rate of the intact lactone ring E could be remarkably reduced if the (20*S*)-hydroxy group is esterified with an oxyalkanoic acid, so that the toxicity of these newly modified camptothecin derivatives could be drastically decreased, but their antitumor activity could be markedly increased. In this study, we synthesized a series of novel (20*S*) camptothecin derivatives (**6**–**15**) with the oxyalkanoic acid esters in the molecules, evaluated their in vivo toxicity, and tested the antitumor activity of these novel compounds against cancer cells in vitro and in vivo (Scheme 1).

Camptothecin esters were prepared in suitable yields by the straightforward acylation of camptothecin with the corresponding oxyalkanoic acid in the presence of a coupling agent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI)[9] and a catalyst 4-dimethylaminopyridine (DMAP) at room temperature. The ^{1}H NMR spectra of these novel camptothecin esters[10] showed the corresponding characteristic protons for their side ester chains.

The cytotoxicity of novel camptothecin derivatives was evaluated on four human cancer cell lines (taxol-sensitive and taxol-resistant) using the colony formation assay.[11] As shown in Table 1, some of newly synthesized camptothecin derivatives were extremely active against four cancer cell lines. Their in vitro antitumor effects were similar to CPT, but significantly higher than that of CPT-11. Furthermore, new camptothecin derivatives of oxyacetic acid proved to be 25–2000 times more effective than Taxol in killing VM46 or MCF-7/ADR cells. These encouraging results suggested that these new derivatives could overcome taxol-resistance in both human colon and breast cancer cells overexpressing multidrug resistant (MDR1) gene. The data also showed that oxyacetate esters (**6**–**12**) ($ID_{50} < 10$ nM) of camptothecin were more effective in killing four human cancer cells (both taxol-sensitive and taxol-resistant) in

Table 1. In vitro and in vivo antitumor activities of novel camptothecin derivatives

	In vitro cytotoxicity (ID_{50}, nM)				In vivo antitumor activity	
	HCT116	VM46	PC-3	MCF-7ADR	MTD/NTD* (mg/kg)	T/C%
Taxol®	2.3	> 50	4.0	> 10,000	40.6	150
Camptothecin	2.8	2.4	3.8	3.9	12	183
Irinotecan	540	220	—	> 500	200	217
6	2.7	0.9	3.5	4.3	150*	217
7	3.6	2.5	6.2	7.2	100*	200
8	3.0	1.8	3.9	4.1	150*	117
9	3.0	1.9	3.2	5.5	150*	300
10	3.5	0.7	3.0	5.2	75*	200
11	3.3	3.0	4.8	7.0	100*	250
12	3.1	2.4	4.6	5.0	45*	200
13	8.0	7.0	—	28	150*	133
14	39	27	—	89	100*	250
15	> 100	94	—	> 100	150*	—

HCT116, human colon cancer cells (taxol-sensitive); VM46, subline of HCT116 (taxol-resistant); PC-3, human prostate cancer cells (taxol-sensitive); MCF-7/ADR, human breast cancer cells (taxol-resistant). All four cell lines were purchased from NCI, NIH. MTD: maximum tolerated dose. NTD*: non-toxic dose. ID_{50}: the drug concentration producing 50% inhibition of colony formation from three point determinations. Standard deviations (SDs) for the in vitro cytotoxicity were less than 10%. For the in vivo efficacy experiments, mice were injected ip with drug solution where drugs were dissolved in cremophor/alcohol (1:1) and then diluted in saline to 5% cremophor, 5% alcohol, 90% saline. T/C% (tumor growth inhibition value) = (surviving days of mice treated with an anticancer drug T/surviving days of control mice C) × 100%.

vitro than oxypropionate ester (**13**) (ID_{50} = 7–28 nM) and oxybutyrate ester (**14**) (ID_{50} = 27–89 nM) and oxyvalerate ester (**15**) (ID_{50} > 94 nM). The shorter the carbon chain between carbonyl and oxygen, the better the antitumor activity in vitro. The in vivo toxicity study showed that the MTD values of new camptothecin derivatives were much higher than CPT and taxol, indicating that all these esters were significantly less toxic to normal mice than parent drug, CPT, and taxol. Compounds **10** and **12** were more toxic to mice than other compounds, suggesting that six-membered intact lactone and methylenedioxy rings of ester chain might increase their in vivo toxicity. The in vivo antitumor effects of these new camptothecin esters were determined on C3H/HeJ mice bearing mouse mammary adenocarcinoma (MTG-B)[12] by a tumor regrowth assay. Surviving days measured from day 0 (when tumor reaches the size of 8 mm diameter) for mice treated with antitumor drugs (T) and surviving days measured from day 0 for control mice (C) were recorded. The preliminary in vivo results showed that all tested compounds (**6**–**13**) were active in controlling MTG-B mouse mammary tumor. The T/C% values of all new camptothecin derivatives except compounds **8** and **13** were significantly higher than CPT and taxol. Among new compounds, the camptothecin-20*S*-(4-bromophenoxyacetate) (**9**) produced the most significant antitumor activity (T/C% > 300). Apparently, the above data strongly suggest that esterification of (20*S*) camptothecin with an oxyalkanoic acid could protect the lactone E ring from hydrolysis or slow down the opening rate of the intact lactone ring E, resulting in reduced toxicity to normal tissues and enhanced antitumor activity. A variety of the substituents on the side ester chains might also affect the anticancer activity and toxicity of these camptothecin derivatives to a certain extent. Further study on the structure–activity relationhsip (SAR) of this new class of camptothecin derivatives is being pursued in our laboratory.

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References and Notes

1. Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888.
2. Hsiang, Y.-H.; Hertzberg, R. P.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.
3. Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1986**, *29*, 1553.
4. Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Tele, C.; Moore, L.; Truesdale, A.; Leitner, P.; Besterman, J. M. *J. Med. Chem.* **1993**, *36*, 2689.
5. Kingsburg, W. D.; Boehm, J. C.; Jakas, D. R.; Holden, K. G.; Hecht, S. M.; Glagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertzberg, R. P. *J. Med. Chem.* **1991**, *34*, 98.
6. Sawada, S.; Matsuoka, S.; Nokata, K.; Nagata, H.; Furuta, T.; Yokokura, T.; Miyasaka, T. *Chem. Pharm. Bull.* **1991**, *51*, 3052.
7. Wani, M. C.; Ronman, P. E.; Lindley, L. T.; Wall, M. E. *J. Med. Chem.* **1980**, *23*, 554.
8. Cao, Z.; Harris, N.; Kozielski, A.; Vardeman, D.; Stehlin, J. S.; Giovanella, B. *J. Med. Chem.* **1998**, *41*, 31.
9. Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.
10. **Ester 6**. Yield 70.2%, mp 210–212 °C, ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H, Ar–H), 8.28 (d, 1H, Ar–H), 7.96 (d, 2H, Ar–H), 7.86 (t, 1H, Ar–H), 7.69 (t, 1H, Ar–H), 7.20 (s, 1H, Ar–H), 7.12 (d, 2H, Ar–H), 7.08 (s, 1H, Ar–H), 6.70 (d, 1H, Ar–H), 5.71 (d, 1H, H17), 5.42 (d, 1H, H17), 5.29 (q, 2H, H5), 4.85 (q, 2H, OCH_2CO), 2.23 (s, 3H, Ar–CH_3), 2.20 (d, 2H, H18), 0.98 (t, 3H, H19). Ester **7**. Yield 68.6%, mp 193–196 °C, ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H, Ar–H), 8.21 (d, 1H, Ar–H), 7.95 (d, 2H, Ar–H), 7.84 (t, 1H, Ar–H), 7.67 (t, 1H, Ar–H), 7.22 (s, 1H, Ar–H), 5.70 (d, 1H, H17), 5.44 (d, 1H, H17), 5.29 (q, 2H, H5), 4.33 (q, 2H, OCH_2CO), 3.20 (m, 1H, OCH), 2.40–2.00 (m, 4H), 2.00–0.60 (m, 19H). **Ester 8**. Yield 81.7%, mp 250–253 °C, ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H, Ar–H), 8.20 (d, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 7.86 (t, 1H, Ar–H), 7.70 (m, 3H, Ar–H), 7.20 (m, 6H, Ar–H), 5.69 (d, 1H, H17), 5.44 (d, 1H, H17), 5.25 (d, 2H, H5), 4.96 (s, 2H, OCH_2CO), 2.25 (dm, 2H, H18), 0.98 (t, 3H, H19). **Ester 9**. Yield 87.1%, mp 232–234 °C (dec.), ^{1}H NMR ($CDCl_3$) δ 8.67 (s, 1H, Ar–H), 8.26 (d, 1H, Ar–H), 8.10 (d, 1H, Ar–H), 7.90 (t, 1H, Ar–H), 7.73 (t, 1H, Ar–H), 7.43 (d, 2H, Ar–H), 7.23 (s, 1H, Ar–H), 6.97 (d, 2H, Ar–H), 5.53 (d, 1H, H17), 5.45 (d, 1H, H17), 5.31 (s, 2H, H5), 5.15 (q, 2H, OCH_2CO), 2.08 (d, 2H, H18), 1.02 (t, 3H, H19). **Ester 10**. Yield 69.8%, mp 147–150 °C, ^{1}H NMR ($CDCl_3$) δ 8.42 (s, 1H, Ar–H), 8.18 (d, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 7.86 (t, 1H, Ar–H), 7.68 (t, 1H, Ar–H), 7.48 (d, 1H, Ar–H), 7.15 (s, 1H, Ar–H), 6.86 (t, 1H, Ar–H), 6.75 (s, 1H, Ar–H), 5.69 (d, 1H, H17), 5.43 (d, 1H, H17), 5.42 (s, 2H, H5), 4.90 (q, 2H, OCH_2CO), 2.31 (s, 3H, $ArCH_3$), 2.25 (d, 2H, H18), 0.97 (t, 3H, H19). **Ester 11**. Yield 57.8%, mp 205–207 °C, ^{1}H NMR ($CDCl_3$) δ 8.42 (s, 1H, Ar–H), 8.28 (d, 1H, Ar–H), 8.20 (d, 2H, Ar–H), 7.98 (d, 1H, Ar–H), 7.88 (t, 1H, Ar–H), 7.72 (t, 1H, Ar–H), 7.19 (s, 1H, Ar–H), 7.02 (d, 2H, Ar–H), 5.68 (d, 1H, H17), 5.42 (d, 1H, H17), 5.30 (s, 2H, H5), 4.96 (q, 2H, OCH_2CO), 5.28 (dm, 2H, H18), 0.94 (t, 3H, H19). **Ester 12**. Yield 53.0%, mp 253–255 °C (dec.), ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H, Ar–H), 8.20 (d, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 7.84 (t, 1H, Ar–H), 7.68 (t, 1H, Ar–H), 7.17 (s, 1H, Ar–H), 6.68 (d, 1H, Ar–H), 6.53 (s, 1H, Ar–H), 6.34 (q, 1H, Ar–H), 5.83 (q, 2H, OCH_2O), 5.63 (d, 1H, H17), 5.39 (d, 1H, H17), 5.26 (s, 2H, H5), 4.76 (q, 2H, OCH_2O), 2.25 (dm, 2H, H18), 0.99 (t, 3H, H19). **Ester 13**. Yield 91.6%, mp 195–198 °C, ^{1}H NMR ($CDCl_3$) δ 8.38 (s, 1H, Ar–H), 8.25 (d, 1H, AR–H), 7.96 (d, 1H, Ar–H), 7.84 (t, 1H, Ar–H), 7.69 (t, 1H, Ar–H), 7.34 (s, 1H, Ar–H), 7.08 (d, 2H, Ar–H), 7.00 (d, 2H, Ar–H), 6.79 (t, 1H, Ar–H), 5.69 (d, 1H, H17), 5.42 (d, 1H, H17), 5.28 (s, 2H, H5), 4.27 (q, 2H, OCH_2), 3.03 (dm, 2H, CH_2CO), 2.25 (dm, 2H, H18), 0.99 (t, 3H, H19). **Ester 14**. Yield 47.3%, mp 185–187 °C, ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H, Ar–H), 8.25 (d, 1H, Ar–H), 7.96 (d, 1H, Ar–H), 7.84 (t, 1H, Ar–H), 7.69 (t, H, Ar–H), 7.34 (s, 1H, Ar–H), 7.22 (d, 2H, Ar–H), 7.02 (d, 1H, Ar–H), 6.79 (t, 1H, Ar–H), 5.69 (d, 1H, H17), 5.42 (d, 1H, H17), 5.42 (d, 1H, H17), 5.28 (s, 2H, H5), 4.04 (q, 2H, OCH_2), 2.17 (m, 4H, H18 and CH_2), 0.97 (t, 3H, H19). **Ester 15**. Yield 77.8%, mp 125–128 °C, ^{1}H NMR ($CDC1_3$) δ 8.39 (s, 1H, Ar–H), 8.16 (d, 1H, Ar–H), 7.94 (d, 1H, Ar–H), 7.82 (t, 1H, Ar–H), 7.69 (t, 1H, Ar–H), 7.23 (s, 1H, Ar–H), 7.19 (d, 1H, Ar–H), 6.95 (t, 2H, Ar–H), 6.90 (t, 1H, Ar–H), 6.77

(d, 1H, Ar–H), 5.67 (d, 1H, H17), 5.43 (d, 1H, H17), 5.29 (s, 2H, H5), 3.97 (t, 2H, OCH_2), 2.62 (t, 2H, CH_2CO), 2.25 (dm, 2H, H18), 1.89 (s, 4H, CH_2CH_2), 0.99 (t, 3H, H19).

11. Yang, L. X.; Wang, H. J.; Holton, R. A. *Int. J. Radiat. Oncol. Biol. Phys.* **2000**, *46*, 159.

12. Experimental procedures for evaluation of in vivo anticancer activity of camptothecin derivatives: The mouse mammary adenocarcinoma cells (MTG-B) was kindly supplied by Dr. Evan B. Douple. The MTG-B tumor was maintained via serial passage in 5- to 7-week-old female C_3H mice (Jackson Laboratories, Bar Harbor, ME, USA). The protocol for MTG-B tumor implantation was described in detail by: Jones, E. L.; Lyons, B. E.; Douple, E. B.; Filimonov A.; Dain, B. *Radiat. Res.* **1989**, *118*, 112.

13. Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. *Cancer Res.* **1989**, *49*, 1465.